REMARKS/ARGUMENTS

Claims 1-13 and 15-25 are pending in the present application and stand rejected on various grounds. No claim amendments have been made with the present response.

Rejections Withdrawn

Applicants note and appreciate withdrawal of the rejections of clams 1-13 and 15-25 under 35 U.S.C. 112, second paragraph.

New Claim Rejections - 35 U.S.C. §103

(1) Claims 1-13 and 15-24 have been rejected under 35 U.S.C. 102(a) as allegedly being unpatentable over Hart et al. (BIO/TECHNOLOGY Col. 12, November 1994) in view of the combined teachings of Wetzel et al. (EP 0155189) and Van Dien et al. (Appl. Environ Microbiol. 1997, 63(5):1689-95).

The Rejection

Hart et al. (especially pp. 1113-115) was cited for its teaching of a process for large-scale production of IGF-I from the periplasm of *E. coli* by culturing *E. coli* host cells having a plasmid comprising an inducible alkaline phosphatase promoter and nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence for secretion into the periplasm.

Wetzel et al. (especially pp. 3-7 and claims 1-9) was cited for teaching a plasmid vector comprising an inducible promoter and nucleic acid encoding a T4 phage lysozyme.

Van Dien et al. (especially Results and Discussion and pp. 1689-1693) was cited for its alleged teaching of genes involved in polyphosphate metabolism in *E. coli* that were cloned behind different inducible promoters on separate plasmids.

According to the rejection, it "would have been obvious to one of ordinary skill in the art at the time the invention was made to place the nucleic acid encoding a T4 phage lysozyme taught by Wetzel et al. behind the arabinose inducible P_{BAD} promoter and/or place the nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence for secretion into the periplasm taught by Hart et al. behind the IPTG inducible P_{tac} promoter," and to "further transform the *E. coli* cells of Hart et al. with the modified plasmid vectors of Wetzel et al. and/or the modified plasmid vector having the nucleic acid encoding a human IGF-I linked to a *lamb* signal sequence

placed behind the IPTG inducible P_{tac} promoter." (Office Action, passage bridging pages 2 and 3) The Examiner adds that the additional steps recited in claim 1, including addition of arabinose after 50% or more of the human IGF-I has accumulated, would also have been obvious to one of ordinary skill in the art at the time the invention was made.

The Examiner finds motivation to make the combination "in order to have synthesis of lysozyme that ruptures the polysaccharide membrane of *E. coli* host cell after accumulation of human IGF-I in the periplasm which simplifies the purification of the human IGF-I." (Office Action, page 3) Alleged motivation to wait until 50% or more of the human IGF-I has accumulated before inducing with arabinose to express T4 phage lysozyme was found in the desire to obtain a greater yield of human IGF-I. Motivation to place nucleic acid encoding the T4 lysozyme and nucleic acid encoding human IGF-I on the same vector is found since this approach "simplifies transformation in the *E. coli* host cell. (Office Action, page 3)

The Examiner further notes that the art of recombinant heterologous protein expression in bacterial host cells "is well developed and widely used in biotechnology for obtaining a desired protein" (Office Action, page 3), and cites Dennis et al. (WO 93/24633, published 12/09/1993) as allegedly showing that lysozyme was important in the purification and recovery of poly-β-hydroxybutyrate from *E. coli* host cells.

The Examiner concludes that "the claimed invention was within the ordinary skill in the art to make and use at the time was made, and was as a whole clearly *prima facie* obvious." (Office Action, page 3) In addressing Applicants' arguments in response to an essentially identical rejection in the previous Office Action, the Examiner refers to *In re McLauglin* for the notion that the kind of hindsight reconstructions made in support of the present rejection is "proper", and refers to MEPE 2143 reciting "[e]xemplary rationales that may support a conclusion of obviousness."

Applicants continue to disagree with the legal standard used, the application of the legal standard and the conclusions drawn by the Examiner, and respectfully traverse the rejection.

The legal standard

In *KSR Int'l v. Teleflex Inc.* 127 S. Ct. 1727 (2007) the Supreme Court stated:

> The combination of known elements according to known methods is likely to be obvious when it does no more than yield predictable results.

Id. at 1739

But the Court also added:

> [A] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.

Id. at 1741.

The Supreme Court stated, in dicta, that under certain circumstances "obvious to try" could serve as a basis for obviousness, but the Supreme Court's analysis and comments were directed to the obviousness of combining *known elements to yield a predictable structure.*

A prima facie case of obviousness has not been established

The process claimed in claim 1 involves the recovery of refractile particles containing a heterologous polypeptide from bacterial periplasm in which the polypeptide is insoluble, using bacterial cells, which comprise (i) nucleic acid encoding a desired heterologous polypeptide including a secretory signal sequence under control of a first inducible promoter; and (ii) nucleic acid encoding a phage lysozyme under control of a second, different, inducible promoter. First, the production of the desired heterologous polypeptide is initiated by inducing the first inducible promoter, resulting in the secretion of the heterologous polypeptide into the periplasm of the bacteria as an aggregate. Once 50% or more of the heterologous polypeptide has accumulated, production of the phage lysozyme is initiated, by inducing the second inducible promoter, resulting in the accumulation of the phage lysozyme in the cytoplasmic compartment of the bacteria. As a result of this coordinated expression schedule, the insoluble refractile bodies are efficiently released from the entanglement in the peptidoglycan layer, resulting in a significant increase in the amount of insoluble heterologous polypeptide that can be recovered from the periplasm of the bacteria by subsequent mechanical disruption of the bacterial cells.

Applicants do not contest that, as evidenced by the cited combination of Hart et al., Wetzel et al. and Van Dien et al, inducible promoters, vectors containing the coding sequences of

heterologous polypeptides under control of inducible promoters, vectors containing the coding sequence of a lysozyme under control of an inducible promoter, and bacterial cells transfected with two separate expression vectors, each containing a different coding sequence under control of a different inducible promoter were known in the art at the time the present invention was made.

However, the invention claimed in the present application goes way beyond this knowledge. As explained in the specification, and as it is clearly reflected in the claims, the process disclosed and claimed in the present application requires the *coordinated expression* of nucleic acid encoding a desired heterologous polypeptide and nucleic acid encoding a phage lysozyme, in which *expression of the phage lysozyme is induced only after about 50% or more of the heterologous polypeptide has accumulated.*

There is nothing in the cited reference, when taken alone or in any combination, suggesting the coordinated expression of a nucleic acid encoding a heterologous protein and a nucleic acid encoding a phage lysozyme, where expression of the latter is induced only after about 50% or more of the heterologous polypeptide has accumulated. Thus, for this reason alone, the claimed invention is not a mere combination of known elements, and, as it will be discussed below, yields more than predictable results.

The Examiner repeats the earlier assertion from the prior Office Action that "one of ordinary skill in the art at the time the invention was made would have been motivated to wait until 50% or more of the human IGF-I has accumulated before inducing with arabinose to express T4 phage lysozyme in order to obtain a greater yield of human IGF-I." (Office Action, page 4) However, just as before, the Examiner fails to provide any evidence in support of this assertion, which is clearly contradicted by the teaching of the specification.

As explained at page 9, lines 29-32 of the specification:

> *"...it would not be expected that induction at the end of a long fermentation process and after substantial product accumulation would produce enough of the phage lysozyme to be effective."*

The Examiner fails to advance any evidence or solid scientific arguments to rebut this teaching, and thus fails to establish a *prima facie* case of obviousness of the invention claimed in the present application.

It is further noted that, the Examiner has failed to adequately address the issue of predictability at the priority date of the present application (October 28, 1998). While recombinant production of heterologous proteins in bacterial host cells was known in the art a decade ago, the success of any particular manufacturing process was far from predictable and depended on a number of variables, as demonstrated by the present invention, which requires the claimed process to be performed in a certain way (e.g., expression of the nucleic acid encoding the phage lysozyme is induced by the addition of an inducer after about 50% or more of the heterologous polypeptide has accumulated, avoidance of using chloroform in any step of the process).

In conclusion, the cited combination of references fails to teach a key element of the present invention, and therefore the present rejection is misplaced and should be withdrawn.

(2) Claim 25 has been rejected under 35 U.S.C. 103(a) over the same combination of references as that cited in the rejection above, and further in view of Balbas et al., Gene 1996, 172(1):65-9. Balbas et al. was cited for teaching the plasmid pBRINT which is characterized as being "an efficient vector for chromosomal integration of cloned DNA into the lacZ gene of *Escherichia coli*." According to the rejection, it would have been obvious to combine the teaching of this reference with the other references cited and such combination would result in teaching the integration of a nucleic acid into the chromosome of a bacterial cell, as claimed in claim 25.

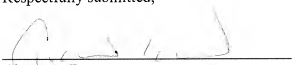
In response to the previous rejection, Applicants have shown that the cited combination of the primary references does not make obvious the invention claimed in claims 1 -13 and 15-24. Since claim 25 depends from claim 1, and Balbas et al. does not provide the teaching missing from the first three references, claim 25 is not obvious either, and the present rejection should be withdrawn.

All claims pending in this application are believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any additional fees for extension of time, or credit overpayment to Deposit Account No. **50-4634** (Attorney's Docket No. **GNE-0128 A (123851-181805)**).

Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,



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